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JOHN A. STEVENSON, - - - - - - - EDITOR,
(CHIEF, DIVISION OF BOTANY AND PLANT PATHOLOGY.)
(*Insular Experiment Station,* - - *Río Piedras, Porto Rico.*)

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EXPERIMENTAL WORK ON THE CONTROL OF THE WHITE GRUBS OF PORTO RICO.

By R. T. COTTON, Entomologist, Insular Experiment Station.

The white grubs of Porto Rico are known to have been present and doing damage on the Island for the last twenty-five years. How serious their ravages were previously we do not know, but since that time they have become gradually more and more destructive, until at present it is impossible to grow ratoon cane in some sections of the Island.

During the year of 1908 the sugar cane in the district of Guánica Central began to suffer very noticeably from the attack of the white grubs, and the following year, matters becoming still worse, strenuous measures were started for discovering methods of controlling this serious pest.

From that time until the present, numerous and varied experiments have been conducted by various entomological workers of the Insular Experiment Station of Río Piedras and the Federal Experiment Station, both independently, and in co-operation with the management of Guánica Central and other centrals of the Island.

No report has ever been published on the results of the vast amount of experimental work conducted along these lines, and although the majority of the results are of a negative nature, they are of considerable interest and some value to entomological workers. Therefore the writer has prepared this brief review.

A considerable portion of the work was carried out by the late Mr. C. T. Murphy, in charge of the experimental work at Guánica, in co-operation with Mr. W. V. Tower, formerly entomologist of the Federal Experiment Station at Mayagüez, and Mr. D. L. Van Dine, former entomologist of the Experiment Station of the Sugar Producers' Association of Porto Rico. Portions of the work have also been conducted by Messrs. Thos. H. Jones and G. N. Wolcott,

former entomologists of the Insular Experiment Station, and by former assistant entomologist Eugene G. Smyth. Mr. R. H. Van Zwaluwenburg, entomologist of the Federal Experiment Station at Mayagüez, has also carried out work along this line, as is noted hereafter. Credit is due to all of these workers for the data given below.

Mr. Eugene G. Smyth, who has recently published an article[1] on the white grubs injuring sugar cane in Porto Rico, states that ten distinct species of white grubs have been segregated and studied. Of these, four belong to the genus *Phyllophaga* and one to the genus *Phytalus* in the tribe *Melolonthini*, while the other five belong to three genera in the tribe *Dynastini*.

It was in connection with the members of the genus *Phyllophaga* that most of the experimental work was conducted and to which the following data apply.

The various experiments have been grouped according to the following headings: soil fumigants, soil insecticides, mechanical methods, poison sprays, and parasitic insects and diseases. Space does not permit the publication of all the experiments conducted in some cases, so selections have been taken that represent the typical results obtained in each group.

SOIL FUMIGANTS.

Under this heading have been grouped the experiments conducted with carbon bisulphide, gasoline, tobacco extract, potassium cyanide, and vaporite, since in all cases the gases or fumes given off by these substances were depended upon to kill the grubs.

Carbon bisulphide.

To test the value of carbon bisulphide as a killing agent, to determine the best method of application, and the most efficient amount of the liquid to use, the two following experiments were made. They were conducted in cane land severely infested with grubs:

Experiment 1.—Use of Carbon Bisulphide in Killing the White Grub.

No. of plot	No. of stools of cane	Amount CS 2 applied	How applied	When examined	Average No. grubs per stool	No. of grubs after treatment		Per cent of grubs killed
						Alive	Dead	
1	4	25cc.	Hole in center of stool.	48 hours later	8	11	21	65
2	4	50cc.	"	"	16	5	60	92
3	4	25cc.	In 2 holes at side of stool	"	15	52	9	14
4	4	50cc.	"	"	10	35	6	14
5	4	25cc.	In 1 hole at side of stool	"	17	52	15	24
6	3	50cc.	"	"	6	25	1	4

[1] Journal of the Dept. of Agric. of P. R., Vol. 1, no. 2., pp. 47–92, no. 3, pp. 141–169.

Experiment II.—Use of Carbon Bisulphide in Killing White Grubs.

No. of plot	No. of stools of cane	Amount CS2 applied	How applied	Depth of injection	When examined	Per cent of grubs killed
1	10	12.5cc.	Center of stool................	5 inches..	5 days later	60
2	"	25 cc.	"	"	"	65
3	"	50 cc.	"	"	"	75
4	"	25 cc.	12.5 cc. each side of stool....	"	"	78
5	"	50 cc.	25. cc. on each side of stool.	"	"	76
6	"	"	In center of stool............	3 inches..	"	90
7	"	"	"	"	"	66

As a result of these experiments it was seen that carbon bisulphide was a good killing agent, and that the best results were obtained by putting the charge in the center of the stools of cane at a depth of about three inches. It was also noted, however, that severe injury resulted to the cane plants from the carbon bisulphide when amounts greater than 12.5 cc. were used.

An experiment was then conducted at Guánica Central, under the direct supervision of Mr. C. T. Murphy, to test out the value of carbon bisulphide on a field scale. Some thirty-three odd acres of land planted to sugar cane were used, arranged in five different sections of 3, 3, 4, 6, and 4 plots, respectively. One check plot was left in each section.

The carbon bisulphide was injected by the use of several "Pal Injecteurs" imported from Germany at a cost of $31.57 each. They were so arranged that any amount of liquid desired could be injected at one time. The only drawback in the use of these instruments was the action of the carbon bisulphide on the rubber connections and leather washers. These very quickly disintegrated, and the small pieces of rubber and leather clogged up the holes through which the liquid was forced. Specially devised rubber connections finally overcame this difficulty, however.

Carbon bisulphide was found to be a most disagreeable and dangerous liquid to handle, for although no serious accidents happened, numerous burns about the hands and face were experienced by the men who applied it.

In all, eight thousand eight hundred pounds of this material were used in the experiment at a cost of six cents a pound. The cost of application was $67.05. The cost of material and labor for the application of fifteen grams per stool of cane amounted to $15.53 per acre: for the application of thirty grams per stool, $31.06 per acre. The following tables give the results of the experiment:

Experiment III.—Results Obtained from Treatment with Carbon Bisulphide.[1]

No. of Section	No. of plot	Amount CS2 applied per stool and how applied	When applied	No. of acres in plot	Weight in tons per acre of cane harvest	Increase or decrease compared with check in tons of cane per acre	Brix	Sucrose	Purity	Tons available 96% sugar per acre	Infestation by grubs [1] at harvest time
1	1	15 grams each side of bank	November 10	.85	38.85	+ 1.41	16.2	12.8	79.0	3.72	Grubs fairly numerous.
	2	Check		.96	37.44		15.6	12.6	80.5	3.56	
	3	15 grams in bank. 15 in furrow	November 10	1.04	35.52	— 1.92	15.7	12.2	77.9	3.21	Grubs very numerous.
2	1	15 grams center of bank	November 10	1.62	43.51	+ 1.46	No mill test taken for this section.				Very few grubs in all three plots.
	2	30 grams center of bank	November 10	1.62	41.12	— .93					
	3	Check		1.62	42.05						
3	1	15 grams in side of bank	November 10	1.95	31.10	— .28	16.3	13.5	82.2	3.20	Grubs very numerous.
	2	15 grams in furrow	November 10	3.54	45.22**	+13.84	17.4	15.3	87.6	5.45	Very few grubs.
	3	Check		1.85	31.38		17.0	14.5	86.6	3.57	
	4	15 grams center of bank	November 10	7.89	33.34	+ 1.96	17.5	15.0	85.4	3.90	
4	1	15 grams center	November 10	.54	31.68	+11.68	16.5	13.1	79.4	3.11	All these plots had an average of 3 grubs per stool.
	2	15 grams each side	November 10	.58	19.72	— .28	17.2	14.2	82.5	2.14	
	3	Check		.61	20.00		16.4	11.5	74.0	1.66	
	4	15 grams center	November 10	1.95	27.27	+ 7.27	15.7	12.7	81.0	2.63	
	5	15 grams bank, 15 grams furrow	November 10	.79	20.12	+ .12	16.6	13.8	83.0	2.13	
	6	15 grams furrow	November 10	.66	28.68	+ 8.68	16.3	13.1	80.1	2.83	
5	1	15 grams center	November 10	.83	30.96	— .41	17.1	14.3	83.6	3.41	Few grubs.
	2	Check		1.03	31.37		16.4	13.4	81.4	3.19	
	3	15 grams in side	November 10	1.71	16.59	—14.78	16.4	13.5	82.3	1.71	Many grubs
	4	15 grams center	November 10	1.44	21.60	— 9.77	17.0	14.2	84.2	2.37	

[1] Canes were planted during September and October of 1910, and were reaped November 15, 1911—January 13, 1912.
** Increase due to better soil.

All plots in this experiment received the same cultural treatment. The only factor that could not be controlled was the condition of the soil. In section four the check plot unfortunately fell upon a piece of bad soil which reduced the yield at least ten tons below the average normal crop per acre. If we disregard this plot, we then find that on six of the ten treated plots the yield fell below the check plots. In all cases where a double dose was applied the yield was lower than on the check plot. The carbon bisulphide had a detrimental effect on the growth of the cane plants.

Altogether the results do not warrant a further use of carbon bisulphide as a means of control for the white grubs in growing cane, for although it may kill the grubs in the stool when first applied the effect is not lasting, and as the egg-laying season of the beetle extends over a rather long period the land soon becomes reinfected and at least three applications a season would be necessary to keep the land free from the grubs. The expense of the material would in any case render such an operation prohibitive. The liquid is dangerous and disagreeable to handle, and furthermore it is injurious to the cane, stunting its growth and often killing the plant.

Carbon bisulphide and gasoline.

In an endeavor to find a mixture that would be cheaper and less injurious to plant life than carbon bisulphide, experiments were tried with gasoline and various mixtures of gasoline and carbon bisulphide. The following table shows the results obtained:

Experiment IV.—Use of Carbon Bisulphide and Gasoline Against White Grubs.

No. of plot	No. of stools of cane	Liquid used	Amount per stool	Where applied in stool	Grubs found after treatment		Per cent grubs killed
					Alive	Dead	
1	10	Pure CS_2	20 grams	Center	6	150	96
2	10	Gasoline	20 "	"	20	147	93
3	10	CS_2—⅓ Gas—⅔	" "	"	10	179	95
4	5	" "	10 "	"	31	57	65
5	5	" "	40 "	"	2	59	97
6	10	CS_2—½ Gas—½	20 "	"	18	127	87
7	3	" "	10 "	"	9	18	66
8	5	" "	40 "	"	6	66	91

All experiments conducted with these two liquids consistently showed that they had almost equal killing powers and could be mixed as desired. However, when applied to young cane all the mixtures

in quantities of twenty grams or more severely burned the plants, and even with mature cane severe injury resulted.

Hence it was found impossible to use gasoline as a substitute or as an adulterant for carbon bisulphide. The great increase in the cost of gasoline at the present time would of course preclude its use, even had it proved to be non-injurious to plant life.

Potassium cyanide.

In view of the deadly nature of potassium cyanide and of reports of its successful use in controlling certain forms of subterranean insects, it seemed desirable to try it out against the white grubs. Experiments were accordingly carried out in which potassium cyanide in both liquid and crystal form was applied in various amounts and at varying depths in the soil, around the roots of the sugar cane.

The potassium-cyanide solution was prepared by dissolving two hundred grams of the crystals in a liter of water. Five grams of the crystals were thus equivalent to twenty-five cc. of solution. For purposes of comparison equivalent amounts of potassium cyanide were used in both experiments. The following tables show the results obtained:

Experiment V.—Experiment with Potassium Cyanide in Liquid Form.

Plot No.	No. of stools of cane	Amount applied	How applied	Depth applied	When applied	When examined	No. grubs found	
							Alive	Dead
1	8	25cc.	Center of stool	3 inches	Feb. 21	March 3	84	13
2	8	" "	"	5 "	" "	" "	87	7
3	7	50 "	"	3 "	" "	" "	39	25
4	7	" "	"	5 "	" "	" "	74	16

Experiment VI.—Experiment with Potassium Cyanide in Crystal Form.

Plot No.	No. of stools of cane	Amount applied	How applied	Depth applied	When applied	When examined	No. grubs found	
							Alive	Dead
1	1	5 grams	Center of stool	5 inches	Feb. 21	Feb. 28	11	2
2	1	" "	"	3 "	" "	" "	27	0
3	2	10 "	"	5 "	" "	March 2	26	8
4	5	" "	"	3 "	" "	" 3	45	18
5	4	5 "	"	5 "	" "	" "	64	2
6	4	" "	"	3 "	" "	" "	68	26
7	5	10 "	"	5 "	" "	" "	78	5
8	5	" "	"	3 "	" "	" "	111	12

From the above tables we see that there was very little difference

in favor of either form of the cyanide as a killing agent. In all cases the injection at three inches gave better results than at five inches. However, neither form of potassium cyanide gave results that would warrant its use as a soil fumigant in controlling the white grub.

The experiments tend to show that the poison has but small powers of penetration when applied in such a manner. The danger incident upon the application of such a powerful poison under existing labor conditions, would in any case render the advisability of using this substance very doubtful.

Tobacco.

Tobacco is an old-time remedy for insect pests, and although it is only effective when used against delicate soft-bodied insects, frequent attempts were made to control the white grubs with it. The following experiment indicates the average results obtained with this substance. Tobacco water was made by steeping eight ounces of cured tobacco in three gallons of water. It was then applied in pint doses to holes in the soil around the cane stools.

Experiment VII.—Experiment with Tobacco Water Against the White Grub.

No. of plot	No. of stools of cane	Amount applied	How applied	When applied	When examined	No. of grubs found	
						Alive	Dead
1	6	1 pint	Hole in center of stool	March 28	April 2	45	2
2	6	1 "	"	"	"	33	3
3	6	1 "	Hole each side of stool	"	"	45	8
4	6	1 "	"	"	"	42	5

It is readily seen that the tobacco in this form had but very little effect on the white grubs. It is too weak in action and too expensive as well to apply in this manner.

Vaporite.

The last substance to be discussed under the heading of soil fumigants is vaporite, a commercial preparation put out in the form of a gray powder, which on coming into contact with moist soil gradually gives off a vapor. In accordance with directions for the most efficient method of using this material, it should be applied at some depth below the root system of the plant treated so that the vapor given off will kill the grubs and other insets infesting the roots as it rises to the surface.

The following table shows the general trend of all the experiments conducted with this substance:

Experiment VIII.—Experiment with Vaporite Against the White Grubs.

No. of plot	No. of stools of cane	Amount applied per stool	How applied	When applied	When examined	No. of grubs found Alive	Dead
1	2	4 oz.	Hole in center of stool	June 8	June 14	6	0
2	4	2 oz.	"	"	"	8	0
3	6	1 oz.	"	"	"	17	1
4	1	2 oz.	"	May 23	May 27	14	0
5	1	2 oz.	"	"	"	20	0

The above results would tend to show that this substance had no effect whatever on the grubs. It is possible that the material had deteriorated somewhat before application.

SOIL INSECTICIDES AND DETERRENTS.

Under the heading of soil insecticides and deterrents have been grouped experiments with a large number of different chemicals and manurial agents in their relation to the control of the white grub. No special order has been observed in presenting them other than to arrange them as logically as possible.

Experiments with lime and various chemicals and chemical compounds mixed with lime.

Experiment IX.—Experiment with Ammoniac[1] and Lime Against the White Grub.

No. of stool	Amount applied	Mixture used	Depth applied	How applied	No. grubs found after treatment Alive	Dead
1	3 ounces	1/3 Lime 2/3 Ammoniac	6 inches	Each side of stool	8	0
2	" "	"	"	"	5	0
3	4 "	1/2 Lime 1/2 Amoniac	" "	"	4	0
4	" "	"	" "	"	4	2
5	8 "	"	" "	"	7	3
6	" "	"	" "	"	5	1
7	" "	"	" "	Center of stool	6	2
8	" "	"	" "	"	8	2

[1] Sal ammoniac (ammonium chloride).

As may be seen by the above table, the ammoniac and lime had little or no effect on the white grubs.

Experiment X.—Experiment with Carbolineum and Lime Against the White Grub.

No. of plot	No. of stools cane	Amount per stool material used		How applied	When examined	No. of grubs found after treatment	
		Carbolineum	Lime			Alive	Dead
1	5	25 cc.	....	In side of stool.......	3 days later.	27	2
2	5	50 cc.	....	"	"	19	0
3	5	25 cc.	4 oz.	"	"	18	0
4	5	50 cc.	4 oz.	"	"	20	1
5	5	25 cc.	....	Each side of stool....	"	21	1
6	5	50 cc.	....	"	"	6	3
7	5	25 cc.	4 oz.	"	"	2	0
8	5	25 cc.	....	In center of stool....	"	3	0
9	5	50 cc.	....	"	"	21	0
10	5	100 cc.	....	"	"	14	1
11	5	25 cc.	4 oz.	"	"	10	0
12	5	50 cc.	4 oz.	"	"	25	0

The carbolineum, both alone and mixed with lime, had apparently no effect at all upon the grubs as a killing agent. Whether or not it had any value as a deterrent was determined in a field trial together with a number of other chemicals. The results are shown in the following table:

Experiment XI.—Experiment with Lime and Combinations of Lime and Other Chemicals Against the White Grub.

No. of plot	Amount and kind of materials used	No. acres treated	Tons yield per A	Brix	Sucrose	Purity	Available tons sugar per acre	No. grubs found per stool
1	Kreso dip 3 gals. Live lime 300 lbs.	.183	31.83	20.5	17.6	85.9	4.45	10
2	20% carbolic acid 5 gals. Lime 500 lbs.	.275	35.36	19.6	15.4	78.6	4.19	10
3	Carbolineum 5 gals. Lime 500 lbs.	.298	40.86	14.2	9.8	69.0	2.75	14
4	100% carbolic acid 5 gals. Lime 500 lbs.	.298	30.45	19.8	15.9	80.3	3.65	13
5	Live lime 250 lbs.	.138	28.08	19.1	15.3	80.1	3.28	14
6	Check	.275	35.82	19.5	16.7	85.6	4.54	15

The sugar cane used in this experiment was planted in January, 1910, and reaped in April, 1911. The lime and other chemicals were applied May, 1910. As is shown by the table, none of the treated plots did so well as the check plot. Plot 3, although higher in yield per acre, was extraordinarily low in purity, but whether this was due to the treatment received or to some other factor, it is difficult to say.

The fact that more grubs were found per stool in the check plot would suggest that some of the chemicals applied exercised a slight deterrent power, but not sufficient to have any practical value.

Experiments with manurial agents.

To test the value of various fertilizers or manurial agents as deterrents of the white grub an extensive series of experiments was conducted. At first, trials were made on a limited scale with beetles in confinement. Flower-pots containing soil and a small amount of fertilizer were placed in a cage with the beetles. Each pot had a different fertilizer mixed with the soil and the beetles had the opportunity to burrow in the pot that was most attractive to them. In this experiment advantage was taken of the habit of the beetles of hiding in the soil during the day. Examination was made of the pots each day and a count of the beetles in each made. Thus at the end of a week the total number of visits per pot was known and some idea of the repellent or attractive powers of each material could be formed.

After a number of these preliminary trials, an experiment was conducted on a field scale with certain of the manurial agents, to determine their effect on the yield and quality of the cane, as well as their deterrent effect as exhibited over an entire growing season. The results are shown in the tabulations that follow.

In the first experiment nine pots of soil, each containing a small amount of fertilizer, were placed in a cage with fifty beetles. The following table gives the amount and kind of fertilizer in each pot, and the number of beetles that visited each pot daily for a week:

Experiment XII.—Repellant Effect of Various Manurial Agents on May Beetles.

No. of pot	Manurial Agent	Amount used	Number of beetles found in pot on the							Total for week
			1st. day	2nd.	3rd.	4th.	5th.	6th.	7th.	
1	Ammonium Sulphate	1 ounce	6	2	3	9	9	12	17	48
2	Potassium Chloride..	"	2	3	1	0	0	2	2	10
3	Potassium Sulphate..	"	4	5	4	5	1	0	4	23
4	Blood..................	"	9	12	11	3	7	21	17	80
5	Cyanamid............	"	0	0	2	7	2	0	0	11
6	Lime	"	0	0	0	0	11	0	0	11
7	Check	"	16	9	11	4	14	0	5	59
8	Bone meal............	"	9	7	13	13	2	5	4	53
9	Phosphoric Acid.....	"	9	12	5	10	4	10	11	61

In the experiment that follows the same materials were used and in the same proportions. However, in this case ten beetles were placed in each pot and at the end of a week the pots were examined and the living and dead beetles in each counted. Thus some indication of the killing power as well as the repellant action was given.

Experiment XIII.—Repellent and Killing Effect of Various Manurial Agents on May Beetles.

No. of pot	Manurial agent	Amount used	Beetles found at end of week	
			Dead	Alive
1	Ammonium sulphate	1 ounce	35	2
2	Potassium chloride	1 "	3	0
3	Potassium sulphate	1 "	6	0
4	Blood	1 "	3	0
5	Cyanamid	1 "	4	0
6	Lime	1 "	0	0
7	Check	1 "	3	12
8	Bone meal	1 "	10	1
9	Phos. acid	1 "	10	1

From the data given in the two preceding tables the following facts are suggested:

Ammonium sulphate does not act as a repellent but shows good killing powers.

Chloride of potash acts as a good repellent.

Potassium sulphate and acid phosphate do not repel but have some killing action.

Cyanamid and lime are good repellents, whereas blood and bone meal are very attractive to the beetles.

Whether or not these qualities hold up under field conditions may be seen in the data given under Experiment XV.

The following experiment was conducted in the same manner as Experiment XII, with the exception that different chemicals were applied to the soil in the pots.

Experiment XIV.—Repellent Effect of Various Chemicals to May Beetles.

No. of pot	Chemical added	Amount used	Number of beetles found in the pots on						Total
			1st day	2d day	3rd day	4th day	5th day	6th day	
1	Lime 9 pts., sulphur 1	2 ounces	0	12	6	2	0	3	23
2	Carbolic acid ½ %	2 "	0	11	8	19	0	1	39
3	Boiled lime sulphur 2-2-50	1 ounce	0	0	6	8	1	8	23
4	Self-boiled lime sulphur 2-2-50	1 "	0	7	6	2	9	3	27
5	Caustic soda 1 lb., sulphur 3 lbs., water 50 gals.	1 "	10	0	6	2	4	5	27
6	Filter-press cake	2 ounces	8	2	6	10	6	7	39
7	Kerosene emulsion 1-8	2 "	20	16	6	7	4	6	59
8	Check		12	2	6	0	26	12	58

With the exception of the kerosene emulsion, there was apparently very little difference in the repellent properties of those chemicals. None of them can be considered as first-class repellents. An experiment with the same chemicals to test their killing powers gave such conflicting results that it has not been included in this report.

The following experiment was carried out to test the value of some of the fertilizers previously referred to, when used under field conditions. Plots of one hundred stools of cane were used for each different fertilizer, and at the end of the season the effect on the yield and quality of the cane, and the number of white grubs was determined.

Experiment XV.—Manurial Agents as Deterrents for White Grub.

No. of plot	Manurial agent used	Amount per stool applied	Yield in tons per acre	Brix	Suc.	Gluc.	Pur.	Average 96% sugar per acr. in tons	Average No. grubs per stool
1	Ammonium Sulphate	8 ounces	26.53	19.4	15.3	2.50	79.0	3.15	11
2	Nitrate of Soda.......	4 "	37.22	19.2	15.8	2.38	82.3	4.45	6
3	Cyanamid	4 "	22.57	20.1	15.4	2.38	76.6	2.55	1
4	Muriate of Potash....	" "	25.74	18.7	16.8	2.27	89.8	3.44	8
5	Potassium Sulphate..	" "	22.97	19.7	16.7	2.17	84.8	2.98	10
6	Acid Phos............	" "	29.30	20.4	17.4	1.78	85.3	3.97	10
7	Lime	1 lb.	25.74	20.2	17.3	2.08	84.7	3.48	14
8	Check		26.93	19.6	16.1	2.17	82.1	3.27	10
9	Kainit................	4 ounces	28.12	21.0	18.4	1.92	87.6	4.08	7
10	Kainit................	8 "	29.30	18.9	15.8	2.27	83.6	3.70	5

From the data presented in the preceding table it is seen that of the materials used cyanamid alone maintained its repellent effect throughout the season. Its effect upon the yield, however, was not good, and the cost of such a treatment, without obtaining a fertilizing compensation to partly offset this cost would make such a practice prohibitive. Further experiments with cyanamid alone have moreover given but poor results and have failed to confirm the results previously obtained.

The nitrate of soda had an excellent effect on the yield and apparently had some slight deterrent power.

Both applications of kainit gave an increase in yield over the check plot and exercised some deterrent effect over the white grubs. None of the fertilizers, however, can be considered as giving results of any very practical value in controlling the white grubs.

Mr. R. H. Van Zwaluwenburg, entomologist of the Federal Experiment Station, has recently carried out a series of four experiments with cyanamid and with cyanamid and acid phosphate, as agents for killing white grubs (*Phyllophaga* spp.) in cane lands. His results are appended herewith.

Summary of Results.

HACIENDA SANTA RITA, GUANICA CENTRALE. APPLIED MARCH 13, 1917; COUNTS MADE APRIL 3, 1917.

	Number of stools	Average grubs per stool
Cyanamid ½ lb. per stool	299	4.1
" 1 " "	91	3.6
No. application (check)	115	3.8

HACIENDA PULIDO, CENTRAL ROCHELAISE, MAYAGÜEZ, P. R., APPLIED OCTOBER 8 TO SIX-MONTHS-PLANT CANE: COUNTS MADE NOVEMBER 23, 1917.

	Number of stools	Average grubs per stool
Cyanamid and acid phosphate 1 lb. per stool	50	3.2
Cyanamid and acid phosphate 2 lbs. per stool	39	3.3
No. application (check)	59	5.1

In this experiment there was so much variation in soil conditions, a ledge of tosca coming out near the surface over much of the field, that not much reliance can be placed on the figures obtained.

At Central Aguirre applications were made in the absence of sufficient grubs to obtain any reliable figures. In one field it was noted that first-instar grubs were present in treated stools, indicating that the eggs are not killed by applications of one pound of cyanamid per stool.

The applications made at Corsica Central were not disturbed. Applications of one pound of cyanamid and one pound of cyanamid and acid phosphate caused no injury at all to young ratoon cane.

Cyanamid alone and cyanamid mixed with an equal weight of acid phosphate are of no practical value against white grubs when applied in amounts up to two pounds to the stool, either when applied as a surface dressing or when worked into the top four inches of soil.

Poison sprays.

Numerous attempts have been made from time to time to kill the beetles by spraying their food plants with arsenical poisons. Since the beetles have biting mouthparts, are hearty feeders and do not fly far, but confine their feeding activities to the immediate vicinity of the cane fields, it seemed highly probable that such methods of control would meet with some success. The arsenicals used in these experiments were arsenate of lead and Paris green. Of the numerous experiments conducted the following four have been selected as being indicative of the results obtained.

Experiment XVI.

Five hundred beetles were caught while feeding on the foliage of young cane. They were placed in a cage and fed for ten days on a common weed, "bleda," (*Amaranthus* spp., a favorite food plant of the beetles), the foliage of which had been sprayed with a solution of arsenate of lead three pounds to fifty gallons of water. At the end of this time the cage was examined and four hundred and sixty-eight of the beetles were found dead while the remaining thirty-two were very sluggish. An analysis of the dead beetles revealed traces of arsenic.

Experiment XVII.

A patch of "bleda" in the vicinity of some cane fields was sprayed with a solution of arsenate of lead of the same strength as that used in the previous experiment. At night five hundred and sixty-five beetles were caught feeding on this poisoned "bleda" and were immediately placed in a cage and fed on fresh, unsprayed material. At the end of a week one hundred and fifteen were found dead, and a week later two hundred more. Analysis showed traces of arsenic.

Experiment XVIII.

This experiment was a repetition of Experiment No. XVI, with the difference that a solution of Paris green was used to poison the "bleda" in place of the arsenate of lead. The solution was made up of one pound Paris green, one hundred and twenty-five gallons of water, twelve and one-half pounds of flour and two and one-half gallons of milk of lime. At the end of ten days an examination revealed two hundred and nineteen dead beetles, two hundred and eighty-one still being alive.

Favorite food plants of the beetles in the fields were sprayed with different strengths of arsenate of lead and Paris green. Beetles were collected at night feeding on these sprayed plants and were kept in cages without food to watch the effect of the poison on them.

Experiment XIX.—Experiment with Arsenate of Lead and Paris Green.

No. of experiment	Host plant used	Poison used	Strength applied	No. beetles collected	Beetles dead after 10 days	Hour of capture
1	Cane	Arsenate of lead	5 ounces. 5 galons	9	0	9 p. m.
2	"	" " "	3 " 5 "	16	2	9-10 "
3	Casuarina [1]	" " "	5 " 5 "	35	15	9-45 "
4	"	París green	15 grams. 5 "	116	23	9-15 "
5	"	" "	15 " 5 "	6	0	10-30 "
6	Salcilla [2]	" "	15 " 5 "	4	0	10-10 "
7	Casuarina	" "	30 " 5 "	96	2	9-15 "

[1] *Casuarina equisetifolia.* [2] *Schrankia portoricensis.*

In this experiment the death rate of the beetles was not greater than would be normal with healthy beetles kept without food. It seems probable in this case that the beetles were captured before they had consumed any considerable quantity of the poisoned foliage.

As a result of these experiments it would appear that the spraying of the food plants of the beetles would undoubtedly cause the death of a portion of the beetles; that the working of the poison is slow and that the beetles would probably crawl to their burrows in the soil before dying; and that arsenate of lead is more effective as a poison for the beetles than Paris green. Unfortunately the practice of spraying large fields of cane and the trees in the vicinity of the fields is too expensive to be practical.

MECHANICAL METHODS.

Use of dynamite against the white grubs.

To test the value of dynamite as an agent with which to destroy the white grubs in infested land an experiment was carried out, in which various charges of dynamite were exploded at different depths in the soil and at distances of five feet apart. Previous to the blasting a careful estimate was made of the number of grubs present in the field. Three areas of eight square feet were selected in different parts of the field and a count made of all the grubs found within those areas. By this method it was estimated that there were one hundred and ninety-six thousand and twenty grubs per acre.

Experiment XX.—Experiments with Dynamite Against the White Grub.

Exp. No.	No. of blasts made	Amount dynamite used per charge	Distance between charges	Depth of charge in soil	Diameter of hole made by blast	Soil left untouched by blast	Per cent grubs killed
1	6	¼ stick	5 feet	8 inches	2 feet	3 feet	52
2	6	¼ "	" "	16 "	" "	" "	52
3	4	½ "	" "	8 "	2½"	2½"	72

As may be seen from the chart, best results were obtained by using a charge of one-half stick of dynamite. However, even that amount exploded at distances of five feet apart left one-half the surface of the ground undisturbed, and in the area that was thrown up by the explosion only seventy-two per cent of the grubs were killed. Many of the grubs were thrown out on the surface of the soil without injury. With charges at five feet apart it would have required one thousand seven hundred and forty-two charges per acre, which would have made the cost per acre, exclusive of fuses, caps,

or labor, $239.46. This, of course, made such a practice prohibitive, even had it been successful in destroying the grubs.

Flooding as a method of controlling the white grub.

In localities where there is an abundant supply of water, it was thought possible that by flooding the infested fields for a certain time that the white grubs might be destroyed. To test this theory the following experiments were made.

Several lots of white grubs were submerged in a tank of water for varying lengths of time. They were then taken out and examined.

Experiment XXI.—Effect of Flooding on White Grubs.

No. of Exp.	No. of grubs used	No. of hours kept under water	Per cent of grubs alive after treatment	Per cent of grubs dead after treatment
1	100	2 hours	96	4
2	100	4 "	100	0
3	50	4 "	100	0
4	15	4 "	100	0

In practically all cases the submergence had no other effect than to make the grubs rather sluggish and limp. They soon regained their normal active condition after being exposed to the air. A further experiment was conducted along these lines to determine whether or not a longer submergence would have different results. Several ditches were plugged at each end and filled with water. A large number of white grubs were then placed in the ditches and left for a period of two weeks. The water was then drawn off and the grubs found to be still alive and active. It would seem from the results of these experiments that flooding would be useless as a method of controlling the white grubs.

Use of light to attract beetles.

Knowing that the majority of insects are attracted to light, attempts were made to destroy the beetles by taking advantage of this natural phenomenon. Arc lights and five hundred candle power Pitner gasoline lamps were used in these experiments, and were run through the beetle season from March to November. The lights were erected over large basins filled with molasses and water, so that the beetles attracted to the lights would fall in and be drowned. In the following experiment two of the lights were erected in the midst of cane fields heavily infested with the white grub, while the third was erected on the roof of a two-story building in the vicinity.

Experiment XXII.—Use of Light Against the Beetles.

No. of light	Where located	Kind of light	Time when run	No. of beetles caught	Average No. caught per night
1	In cane field.....	Arc light.........	Nightly from April 20 to May 31.	250	6
2	"	"	"	65	1.5
3	On roof of bldg. near cane field	500 c. p. Pitner gasoline lamp	Run for 23[1] evenings in July, Aug., Oct., and November	24	1

[1] On only 7 nights were May beetles taken.

The results of these trials clearly show that little success can be expected with light as a means of attracting the beetles. Since these experiments were conducted it has been found that *Phyllophaga* beetles of the Island are ordinarily attracted to light only during their flight to their food plants in the early part of the evening. The flight only lasts about an hour, and once they have started feeding the beetles are no longer attracted by light. Hence it would be useless to run the lights for more than an hour each night.

Collection of the grubs and beetles.

The most successful method of controlling the white grub that has yet been found is that of collecting the grubs and beetles. The method is rather expensive but it is the only sure way of keeping the pest from increasing.

Some idea of the expense may be obtained from the following figures supplied by Guánica Central, where this method is practiced. During seven months in 1914, a total of 2,255,000 beetles and 1,662,000 grubs were collected at a cost of $2,710.60. The following year 2,468,000 beetles and 2,425,000 grubs were collected at a cost of $3,443.77. More of the grubs and beetles are being collected each year by this central in an endeavor to reduce the numbers of this pest, but unfortunately the method is not very generally practiced by the cane growers of the Island, and in fact the majority of the growers use no method of control whatsoever.

Collections of the grubs should be made when the land is being plowed. Large numbers of the grubs are turned up at this time and should be collected by boys and women following the plows. Where turkeys, chickens, and hogs are available they should be turned into the fields at plowing time, as they will find and devour those grubs that the pickers fail to see.

The beetles feed at night on the foliage of the cane and the trees in the vicinity of the cane fields. They are rather sluggish in their

movements and may be readily captured. Boys and men can go around at night with bags and lanterns and collect the beetles in large numbers by shaking them from their food plants.

The beetles fly during the months of March to November, inclusive, but they are more abundant and hence more easily collected at certain times during this period. These periods of abundance vary somewhat with the locality and it is necessary to watch conditions carefully in order to take advantage of them.

INSECT PARASITES AND FUNGUS DISEASES.

In view of the fact that the white grubs of Porto Rico suffer but little from the attacks of insect parasites, attempts were made to introduce some from other parts of the world. Reports on these efforts have been made in other publications of this station, so that no further mention will be made of this work here.

In addition to the introduction of insect parasites attempts were made to utilize a disease of the grubs and beetles known as the green muscardine fungus, *Metarrhizium anisoplia*. A report on this work has been made by Mr. John A Stevenson, pathologist of this Station, and may be found in this number of the *Journal*.

CONCLUSION.

In conclusion it may be stated that control of the white grub is still one of the largest entomological problems of the Island and that undoubtedly a vast amount of work still remains to be carried out. The collecting of the grubs and beetles is at present the only practical method of holding them in check, and it is far from being entirely satisfactory.

It seems likely that the most promising road to success in white-grub control for Porto Rico will be in the introduction of predacious and parasitic enemies.

THE GREEN MUSCARDINE FUNGUS IN PORTO RICO.

(*Metarrhizium anisopliæ* [Metsch.] Sorokin.)

By John A. Stevenson, Pathologist, Insular Experiment Station.

The green muscardine fungus is one of the best-known of the fungi attacking injurious insects, and as such has been studied and observed in many parts of the world. It occurs apparently indigenous in some countries, and has been introduced into various others in an endeavor to make use of it in the fight against insect pests. It is probable that with the exception of the work with *Sporotrichum globuliferum*, an enemy of the chinch bug and other insects, more has been done with this fungus than with any other of a similar nature. Although originally described from Europe, most of the studies upon it and attempts at its artificial dissemination have been made in the tropics or subtropics and in connection with sugar-cane insects.

HISTORY OF THE FUNGUS.

The fungus was first noted and described by Metschnikoff (23)[1] in Russia in 1879 as *Entomophthora anisopliae*. Since that time it has been redescribed under several other names or new combinations.[2] Rorer (29) has given a full account of these nomenclatural details. The combination adopted by him in 1910 has been used by other workers since that time, and is also used in this paper.

Since the original discovery in Russia, *Metarrhizium* has been found occurring naturally in France (6), United States (25), Mexico (40), Trinidad (4, 12), Samoa (8), Philippine Islands (2), Queensland (38), Java (26, 41), Hawaii (16, 17), and Porto Rico. In addition the fungus has been introduced for trial under artificial conditions into Mauritius (5), Java (11, 31), Porto Rico (42), Cuba, and Argentina (7). Cultures from this laboratory have been sent

[1] Figures in parenthesis refer to literature cited, p. 28.

[2] *Metarrhizium anisopliae* (Metsch.) Sorokin.
Entomophthora anisopliae (Metsch.) (23).
Metarrhizium anisopliae Sorokin (33).
Isaria destructor Metsch. (24).
Oospora destructor Delacroix (6).
Penicillium anisopliae Vuillemin (43).
Septocylindrium suspectum Massee (22).
Chromostylim anisopliae Sorokin (?).
Penicillium anisopliae Vuillemin (43).

to Java and the two latter countries. Speare (34) reports that no introductions were made into Hawaii from Japan or other sources, as far as the available records show, although statements to the contrary have been made.

HISTORY OF THE INTRODUCTION OF METARRHIZIUM INTO PORTO RICO.

The following paragraph from Bulletin 10 of this Station gives the history of the introduction of *Metarrhizium* into Porto Rico:

"*Metarrhizium* was introduced under the name of Hawaiian beetle fungus by D. L. Van Dine, January 12, 1911, and was identified by the writer, whose identification was confirmed by Prof R. Thaxter. * * * This material consisted of infected beetles, some of which were sent to Mr. C. T. Murphy at Guánica Central. More fungus in the form of infected soil was received by Mr. Van Dine, March 30, 1911. On June 3, a letter accompanying material was received at the Sugar Planter's Station" (now the Insular Experiment Station) "from Mr. D. W. May of the Mayagüez Experiment Station. This material was originally from Hawaii."

It was supposed at that time that the fungus was not indigenous, but since then the finding of infected insects in regions distant from the points where the introduced material has been worked with, makes it appear that it did exist in the Island previous to the Hawaiian importations. The native type has at no time been abundant, having been found on single, isolated insects only.

THE FUNGUS.

Although the fungus has been placed at different times in such widely different genera as *Entomophthora, Isaria, Oospora,* and others, a study of actual material leaves no doubt as to its position near *Penicillium* in the *Moniliaceae*. With one exception, no spore form other than the chains of cylindrical conidia have been reported. Tryon (38) from Queensland makes mention of having found a *Cordyceps* or perfect stage associated with *Metarrhizium*. He assumes the two to be stages of the same fungus, but apparently made no careful cultural studies to prove this assumption, or at least none are given. In as much as no other instances have been recorded. in all of the many references to the subject, of any other spore form such a possibility seems remote. In Porto Rico the fungus has been studied in the laboratory, insectary, and in the field by practically all of the various men who have been connected since 1911 with the

divisions of Pathology and Entomology, and there has never been the slightest evidence to support a theory of another stage in the life history.

Mr. Johnston (14) records two conidial forms, forma *major* and forma *minor* occurring on different hosts. Sufficiently complete accounts of the appearance and behavior of the fungus on artificial media as well as technical descriptions will be found in several of the articles cited in the bibliography, in particular those by Rorer (29), Speare (35), Johnston (14), and the earlier papers by Metschinkoff (23, 24).

HOSTS.

The number of species of insects attacked is very large, and includes many that are of considerable economic importance. A list of the more important host species, together with the country from which the report was made, follows:

Adoretus compressus, Java (41).
Adoretus tenuimaculatus, Hawaii (16–18).
Adoretus umbrosus, Hawaii (35).
Agriotis manci, New York (25).
Anisoplia austriaca, Russia (22).
Anomala, sp., Hawaii (35).
Cleonus punctiventris, Russia (32).
Cyrtacanthracris nigricornis, Java (31).
Holotrichia helleri, Java (11).
Lachnosterna sp., Illinois, reported by Wolcott.
Lepidiota albohirta, Queensland (38).
Leucophilus rorida, Java (31).
Oryctes rhinoceros, Samoa (8).
Phytalus smithi Mauritius, (?) (5).
Rhabdocnemis obscura, Hawaii (29).
Tomaspis postica, Mexico (40).
Tomaspis varia, Trinidad (9, 28).

It will be noted that this series includes the froghopper of Trinidad, as well as a number of other serious cane pests; the rhinoceros beetle, a pest of the coconut; the cockchafer of Europe, and other well-known depredators. The silk-worm has also been reported as a host by Delacroix in France (6).

A considerable range of hosts has also been found in Porto Rico but for the most part only insects in confinement in the breeding cages. These have been collected by the several entomologists of

the Station, but more particularly by Mr. E. G. Smyth (32) who carried on work with the white grubs over a number of years.

The insects found diseased by *Metarrhizium* in Porto Rico are as follows:

Aphodius sp.
Canthon sp.
Dyscinetus barbatus.
Lygyrus tumulosus.
Metamasius hemipterus.
Phyllophaga citri.
Phyllophaga guanicensis.
Phyllophaga portoricensis.
Phyllophaga vandinei.
Phytalus insularis.
Strategus titanus.
Tiphia inornata. (Received from Illinois.)

The fungus has been found in addition on a number of undetermined Scarabaeids, an earwig, a roach, and some other unnamed hosts. The localities of the many collections made have been Río Piedras (Experiment Station), Santa Rita (Guánica), and Añasco, localities into which the fungus was introduced. Specimens have also been taken at Fajardo (*Phyllophaga* sp.), Hacienda Santa Isabel of Aguirre (*Phyllophaga* sp.), and Sierra de Naguabo (earwig), localities into which the fungus was not introduced, apparently tending to prove that the fungus is indigenous to the Island.

Further information on the stages of the various hosts attacked, prevalence, progress of the disease on the individual insects, and related points will be found in the report by Smyth (32).

EXPERIMENTS IN ARTIFICIAL DISSEMINATION.

Metschnikoff (23) conducted the first experiment with *Metarrhizium,* using it to fight the cockchafer of wheat. He obtained his spore material from infected insects. Krassilstchik (19, 20) used the fungus in a similar fashion in this work on the sugar beet curculio and reported from fifty to eighty per cent of the insects infected. These earlier experiments were necessarily on a limited scale because of the difficulty of obtaining spore material in quantity.

During recent years extensive tests have been carried out in Java, Hawaii, Trinidad, Porto Rico, and other regions.

The most important of the efforts in this connection has been Rorer's (27–29) work in Trinidad directed against the froghopper

(*Tomaspis varia*). Entomologists and others (1, 3, 4, 9, 10, 13, 39) connected with the agricultural work of that Island have also assisted in the tests. In the preliminary experiments adult froghoppers in wire cages were inoculated by spraying, and a high percentage of mortality resulted. Results were also obtained in infecting the nymphs. A field experiment, using a mixture of flour and spores, in which over one hundred cane plants were dusted, resulted very favorably in the death by the fungus of a large number of the insects.

Work was then commenced on the production of spore material in great quantities so as to permit of the dusting or spraying with spores of entire fields. For this purpose large cabinets capable of being sterilized by steam were devised. The manner of construction of these cabinets and the course of procedure in producing the spore material have been fully described by Rorer. The results obtained were sufficient to cause these spore-producing plants to be erected on a number of the sugar estates. These are operated at such times as conditions seem favorable for rapid increase of the froghoppers.

The experiments in Java have been carried on in several sections of the Island by different workers but have all been confined to tests on a small scale, mostly in breeding cages apparently. Groenewege (11) infected soil with the spores and then added larvæ of various insects, varied proportions of which were killed by the fungus. He concludes that since most of them were killed near the close of the larval period, and after the full damage to the host would have been caused, that the method is not efficacious. He furthermore stated that the cost would be prohibitive for field operations.

Rutgers (31) carried out experiments at practically the same time, using cultures obtained from Hawaii. For infection he employed spores mixed with a double quantity of flour. The insects used were *Leucophilus rorida* and locusts (*Cyrtacanthacris nigricornis*). In one experiment a mortality of eighty per cent was obtained, but succeeding tests gave only slight results. It was found that infection, even when the insects were enclosed in a small space and were in intimate contact with quantities of spores, was dependent upon external conditions, particularly the moisture content of the air. For this reason and since *Metarrhizium* is found under natural conditions attacking a wide range of insects he concluded that it is a dangerous parasite only under favorable conditions, and that attempts to spread the fungus artificially would be useless.

Speare (35) in Hawaii carried out an extensive series of laboratory inoculations with the fungus, working with *Rhabdocnemis obscura* as the host. The mortality varied considerably in the different trials, not exceeding sixty per cent, however, in any case. No field tests are reported.

Tryon (38) has also conducted tests upon the parasitism of *Metarrhizium*, using the spores mixed with a fine soil rich in organic matter. Final results and conclusions are not given beyond the statement that the fungus appears to have possibilities.

In Samoa (8) excellent results were reported in controlling on an extensive scale the rhinoceros beetle, an enemy of the coconut. Infected beetles were placed in trap piles of rotten coconut husks and other debris, scattered about through the coconut groves. The beetles gathered in these piles for egg laying, and it was claimed that practically all the larvæ were ultimately attacked and killed by the fungus.

Porto Rico.

Work with this fungus was begun immediately upon the receipt of material from Hawaii. Infected beetles and soil containing spores were sent to Mr. C. T. Murphy in charge of experimental work at Central Guánica. In June, 1911, he reported as follows: "The Hawaiian beetle fungus seems to be working well and the beetles kept under control in the cages seem to take it up rapidly At present several thousand are under control and in a few days time, I shall start letting them loose in the fields * * *. I am also propagating the spores artificially so as to more thoroughly infect the beetles." About a year later (April. 1912) he reports that "Inoculating beetles with the Hawaiian fungus started earlier in the month. The fungus took readily and appears to be increasing in virulence; about a fortnight after inoculating the cage, eighty-seven beetles were found killed by the disease. During the next month liberations of dead and sickly beetles will be made about every ten days in a field where the work can be watched."

Mr. Murphy stated that in May he found evidence of beetles having been killed by the disease. and in July a final mention of Metarrhizium occurs in his reports to the effect that "Beetles infected with the Hawaiian fungus have been liberated during the month in cane fields. and at the roots of trees around the nursery

beetles killed by the fungus have been buried so as to infect the soil." It is not apparent that any practical results came from this work.

At the Experiment Station at Río Piedras in connection with cultural studies of the fungus Mr. Johnston, then pathologist, carried on in 1912 a series of inoculation tests in screened cages. Beetles and larvæ (*Phyllophaga vandinei* for the most part) were obtained from the vicinity of Añasco, and Santa Rita, Guánica. These were stored in three cages until transferred to the inoculating cages. It may be noted that ten beetles were found infected in these supply cages as the transfer was being made, pointing again to the natural occurrence of the fungus.

In addition to the *Metarrhizium* trials, other entomogenous fungi secured from France were used in a similar fashion. These were *Sterigmatocystis ferrugineus*, *Sporotrichum globuliferum*, *Isaria densa*, and *Botrytis Bassiana*. No positive results were obtained with these, but on the other hand the beetles in each of the boxes in which these fungi were employed showed infection with *Metarrhizium*, as will be noted hereafter.

The *Metarrhizium* material used was a transfer from an isolation made from infected insects received from Hawaii. In each case the fungus material was scraped off the surface of the medium (yam cylinders) and placed with distilled water in an atomizer. The surface of each box was thoroughly sprayed with the spore suspension, and the culture medium itself placed on the surface of the soil. The beetles were then added from the supply boxes and observations taken from time to time. All beetles found dead were held for full development of any fungus that might be present, so as to permit of exact determinations.

As the results obtained were much the same for all, details of the examination of but one box are given, as per the following table:

Infection of May-Beetles by *Metarrhizium* (Box No. 1).

Date	May 24	June 1	6	10	15	21	26	July 1	8
Dead on surface.	1			6		5	2		
Infected.........						1			
Dead in soil.....	9	18	17	16	7	9	3	5	2
Infected.........	5	8	8	6	3	3	1	2	1

Total dead, 100: infected by *Metarrhizium*, 38.

A Summary of results from all the boxes follows:

Infection of May-Beetles by *Metarrhizium*.

Box No.	Inoculated with	No. of beetles dead	No. infected with *Metarrhizium*	Per cent infection
1	Metarrhizium........	100	38	38
2	"	113	22	19
3	Sterigmatocystis.....	98	35	35
4	Sporotrichum........	102	18	17
5	Check...............	97	29	29
6	Supply...............	628	93	14
7	Isaria................	92	31	33
8	Botrytis..............	104	29	27
9	Supply...............	1132	190	16

It can very clearly be seen from these results that the fungus was, first of all, not especially virulent toward the May-beetles and that above all it occurred independent of inoculatons.

In order that thorough field trials might be carried out, there was constructed at the Station an apparatus (see Fig. 1) consisting of two cabinets and a five-horse-power upright boiler, following the plans of Rorer (27, 30). The medium used was rice, spread out in thin layers on the shelves and cooked *in situ*. No particular difficulties other than mechanical ones were encountered, and the first batch of spore material was taken off by Mr. Johnston in August, 1913. A low-grade flour was used to dilute the spore mass and to make removal from the rice media more easy.

The resulting material consisting of about fifty pounds of the flour-spore mixture and a similar amount of the rice residue was taken to Yauco on the south coast, and applied to one of the fields of the Guánica Central. About an acre of young plant cane was dusted, using two types of hand dusters, the Furet and the Cyclone. The former was the more convenient and serviceable. The rice residue material was applied by hand around stools of cane adjoining the dusted area.

In October a considerable number of adults were collected from the dusted area by Mr. Smyth and confined to Santa Rita. Only one of the entire number showed at any time signs of *Metarrhizium*.

The following year another lot of spore material was prepared by the writer and again applied to the field previously dusted at Yauco and in the same manner. It has never been apparent that any infection resulted among the beetles in this field. A third lot

of material was some months later applied at Río Piedras not only to cane, but around the bases of a number of coconut trees, which had been severely attacked by *Phyllophaga* sp. Infected insects have never been recovered.

CONCLUSIONS.

As a result of the field observations and the varied experiments carried out by the members of the Station staff and others who have been connected with the project, the conclusion seems justified that the green muscardine will not serve as a practical means of controlling the white grubs or May-beetles in Porto Rico.

It is true that in confinement various stages of *Phyllophaga* spp. are subject to attack, as are also other cane pests, but even in these instances the disease has not been virulently parasitic. No positive results have been obtained in the field tests and it appears that the fungus is indigenous, but so dependent upon humidity and other natural conditions that it is a negligible factor in controlling insect pests and will remain so.

This conclusion is borne out by the reports of workers in Java, both Rutgers (31) and Groenewege (11) stating that while considerable numbers of insects were attacked in confinement, results in the field were so absolutely dependent upon the weather that no artificial attempts at dissemination of the disease would avail. The favorable results obtained in Samoa in the control of the coconut beetle may be easily accounted for by the fact that conditions approximated confinement, the piles of debris retaining moisture, so that for all practical purposes they were no different than so many insect cages. None of the Porto Rican insects lend themselves to this method of trapping.

In Trinidad most favorable results have been reported, it is true but in this case the insect pest involved has an entirely different mode of life from the May-beetles, which it is thought will account for the difference in the efficiency of the fungus in the two regions. It is also quite possible that the weather conditions prevailing at times of severe froghopper infection may favor the fungus.

It does not seem advisable to carry out any further work with the green muscardine in Porto Rico, at least in connection with the white-grubs or May-beetles.

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PLATE I.

THE GREEN MUSCARDINE FUNGUS IN PORTO RICO.

FIG. 1.

FIG. 2.

FIG. 3.

EXPLANATION OF PLATE I.

Fig. 1.—Cabinets used in the production of *Metarrhizium* spore material and boiler, the source of the steam supply.

Fig. 2.—Pupa of *Ligyrus tumulosus* infected with *Metarrhizium*, showing also molted larval skin.

Fig. 3.—Pupa of *Strategus titanus* infected with *Metarrhizium*, showing characteristic conidial masses.

Figs. 2 and 3 from photographs by Smyth.

STUDIES IN INHERITANCE IN SUGAR CANE.

By H. B. Cowgill, Plant Breeder, Insular Experiment Station.

INTRODUCTION.

Although only a few experiments have been conducted in the breeding work with sugar cane at this Station, for the sole purpose of studying inheritance and related subjects, it has been the purpose, as the work progressed, to secure as many useful data as possible from the seedlings which were being propagated and selected. Points which are of interest are the extent to which characters are inherited from the parent varieties when the latter are self-pollinated: whether new types are produced in the nature of mutations: and in what manner and to what degree varieties can be expected to transmit their characters to seedlings when crossed. Various cane varieties have been tried as seed-producers, and it has become evident that, in general, when cross-pollination has not been attempted, there is clearly a difference in the appearance and apparent value of seedlings produced from the different varieties, and that there is also considerable difference in the amount of resemblance to the parent varieties. In many cases this resemblance is plain, and in others there is very little similarity. The reason for this may be that all cane varieties are probably more or less heterozygous, the cases of closer resemblance being due to more homozygous parentage. It is also possible that accidental intercrossing sometimes takes place between varieties growing in the same locality, and that this affects the appearance of the resulting seedlings. Resemblance to both parents has also been observed, when cross-pollination has been affected between varieties, and it is worthy of note that in some cases similarity to the pollinating parent is very plain.

SEEDLINGS SHOWING RESEMBLANCE TO SEED PARENT.[1]

A very close resemblance of seedlings to parent cane, as to visual characters, has been observed every year since 1913 in seedlings pro-

[1] For descriptions of cane varieties see "A Method of Identification and Description of Sugar Cane Varieties and its Application to Types Grown in Porto Rico"; H. B. Cowgill, Plant Breeder, Insular Experiment Station, Porto Rico: Journal of the Department of Agriculture, Vol. 1, No. 3, July, 1917.

duced from seed of D–109.[1] This is a dark greenish-red to purple cane, usually reclining in habit, with buds before expanding semi-elliptical in shape. Many of the seedlings plainly show some or all of these characteristics, while the resemblance as to color is especially noticeable.

A great majority of the seedlings of T–77 are very much like this variety in color and habit, and resemble it more or less as to the form of the bud.

Out of thirty-four seedlings produced in 1916 from B–347, a light-colored cane, only two were of a darker shade than the parent, and seventeen of them showed spots on the internodes more or less like the characteristic spots on the parent variety.

The B–109 seedlings produced in 1916, one hundred and ninety-two in all, were all yellowish-green in color, being like the parent variety in this respect. They also resembled the parent more or less as to the shape of the internodes and the buds. One seedling was darker green than the parent variety and had a tinge of red on the upper internodes; one was a shade darker green, but without the reddish tint; three were greenish-yellow like the parent, but tinged with red on the upper internodes; three were the same color as the parent with the addition of brownish-red blotches on the stalks.

Other variations occurred among these seedlings as follows. Two were markedly glaucous; one had especially prominent buds; one had extremely short joints; two had many adventitious roots; one had especially thin stalks; one was very thick-stalked. There was also a great difference among these seedling as to vigor of growth. Those growing in the area of better soil were taller and of larger girth, while a majority of those on poorer soil appeared more or less stunted, some of them producing almost no stalks.

Out of three hundred and sixty-six seedlings from the variety D–448, which is a red cane, two hundred and twenty-one or sixty per cent, showed redness on the stalk, though some in a less degree than the parent variety. Thirty-four per cent were red all over the stalk, but some were a lighter red than the parent cane. Twelve per cent were as dark or darker than the parent.

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[1] These seedlings and those of subsequent instances cited, except where cross-pollination is indicated, were raised from seed from open-pollinated tassels. For that reason the purity of the pollen can not be guaranteed. However, it does not seem probable that sugar-cane pollen is carried more than a short distance by the wind. It has no special adaptation for being transported and is soft and delicate. In some cases observed the stigmas of the florets were in close contact with the dehiscing anthers. The anthers are shed in great numbers, and possibly they pollinate other florets as they fall. For these reasons it seems probable that the tassels in the center of a field of a pure variety are, without exception, pollinated by pollen of the same variety. The similarity of the seedlings in many cases also tends to verify this belief. It is planned to bag tassels for self-pollination to obtain further data on this point.

Among these seedlings two non-glaucous wine-colored canes occurred; two were greenish-yellow; two were reddish-green and glaucous; three were light reddish-green and glaucous; one had distinctly tumid joints.

All seedlings produced from D–117 seed have shown marked resemblance to this variety in color and in habit of growth, but they have shown more variation in the type of the internode and the bud. Abnormalities such as dwarfed canes, extremely short internodes, wedge-shaped internodes, and buds of unusual form have been common. In using the term abnormality the writer includes only stools distinctly different from the varieties cultivated for commercial purposes, and especially those unfit for commercial cultivation.

Approximately nine hundred D–117 seedlings were grown to maturity in 1916–17. In color they were almost uniformly like the parent variety. No dark-colored canes whatever were found among them. One seedling only out of this number was a slightly different color, being green instead of yellowish-green. The most marked variations were in length of stalk and length of internode. Some of the stools were reclining in habit, but most of them were as erect-growing as the parent variety.

In all, twenty-four abnormal stools were found among these seedlings.[1] Nine of them were classified as "dwarfs." They had stalks not over three feet long and almost uniform in length; internodes one-half to one inch long; usually semi-prominent buds; erect-growing leaves; and often few or many shoots growing from the base of the stool. Some of the abnormal cepas were similar to the dwarfs, but had one or more long stalks.

Other unusual characters in these abnormal canes were stalks with all or many of the buds sprouted, and stalks with many adventitious roots. Still other unusual characters, especially among the dwarfs, were the presence of dead stalks in the stools and a tendency for the entire stool to have withered or weak tops. Some stools also had stalks with wedge-shaped internodes, each averaging about an inch long on one side of the stalk, and narrowed down to sometimes practically nothing on the opposite side. It is planned to grow some of these variations to see whether the abnormal characters are inherited.

[1] It might be assumed that the unusual types which are found among cane seedlings are due to characters acquired by intercrossing of various types of cane at an early stage in the development of the species, and that these characters have been hidden by reason of the dominance of others, since cane has been propagated by sexual means for an unknown period of time. But the question also presents itself whether such abnormalities are not of the character of mutations, and whether some of the other variations in cane seedlings may not also belong to the same class.

RESEMBLANCE OF SEEDLINGS TO PARENTS IN CANES FROM CROSS-POLLINATED TASSELS.

Seedlings produced from tassels of Crystallina cane, which had been bagged and pollinated by D–109 in 1916, showed resemblances to both parent varieties. Some of them were almost identical in appearance with the pollinating variety, while a few closely resembled Crystallina. Between these two types many variations could be found.

The method followed in crossing is described in the Fourth Annual Report of this Experiment Station.[1] A bag of closely-woven cheese-cloth is supported by means of a bamboo pole over a tassel of a variety which is, for practical purposes, pollen-sterile. Cut tassels of the variety which is to furnish the pollen are tied in position inside the bag, so that the wind will carry the pollen, as it is shed, to the stigmas of the tassels of the other variety. This method has proven very satisfactory for our purposes, as a large number of crossed seedlings are produced, and there is very little possibility of any pollen fertilizing the ovaries of the female parent tassel except that from the tassels introduced into the bags, or occasionally that from its own anthers. If the variety used for a pollinator happens to be a dark-colored cane and the other lighter colored, as was the case in this cross, it is then possible to observe many seedlings which show this character of the male parent. There is then little possibility of doubt but that they are the result of cross-pollination. Where the parents are not so distinctly different it is impossible to be absolutely certain whether individual seedlings are from cross-pollination or from self-pollination of the variety intended for the seed parent. The progeny taken as a whole, however, can be considered largely cross-pollinated seedlings.

The canes resulting from cross-pollination last year have grown well and many of them appear promising for commercial culture. Only one abnormal stool was found among them. This was a very small stool, the stalks being only about one-fourth inch to one-half inch in diameter and proportionately short. The leaves were also relatively small. Except for its size this cane was quite like D–109, the variety used as a pollinator.

The following types were observed among these seedlings:

1. Typical D–109.
2. Typical Crystallina.

[1] Fourth Annual Report, Board of Commissioners of Agriculture of Porto Rico, pp. 22–33, 1914–15.

3. Like D–109 as to color but with internodes and buds like Crystallina.
4. Like D–109 in shape of internodes and buds, but of a lighter color and very glaucous.
5. Canes greenish-red to wine color, very little glaucousness, with buds more resembling those of Crystallina than D–109, and with internodes intermediate between the two.
6. With internodes like those of D–109, color like Crystallina, but larger in diameter than either.
7. With internodes and buds like Crystallina, but the color darker and somewhat like that of D–109.
8. Like D–109 as to internodes and buds, but like Crystallina in color.
9. Like D–109 in color but with large nodes, constricted internodes and with buds somewhat like those of Crystallina.
10. Like Crystallina as to buds and internodes but more glaucous.

The following data concerning these canes were also noted:

	Number.	Per cent.
Total stools	560	100
Stools as red as D–109	147	28.3
Stools darker than Crystallina	294	52.5
Stools having internodes like D–109	246	43.9
Stools having no character like D–109, and with color and internodes like Crystallina	42	7.5

These observations were made when the canes were quite mature, so that there was the least possible chance of subsequent change in appearance; but cane varieties are extremely variable and their appearance is affected in many ways by outside influences, such as soil conditions, moisture and sun-light, so that it is difficult to separate different types. Should varieties still more distinctly different be chosen for crossing, more definite results would possibly be secured in the resulting seedlings. The above data, however, show certain points which are worthy of note. They indicate that there is a form of combination of characters in some, at least, of the seedlings resulting from a cross between two varieties of cane. This may be due to certain characters derived from each parent variety, being dominant in the heterozygous seedlings. It also appears that there is greater variation in seedlings so produced, than in those obtained from tassels not cross-pollinated.

As to the economic value of the seedlings produced by crossing,

there is little to be said at present. Many produced by this cross appear very promising, and a relatively large number were selected for extension and further trial, although their true value will not be definitely known until they have been tested further.

Available data as to the sucrose content of the juice of three of the groups of seedlings under consideration, as well as of two other groups which were germinated in 1912, are given here. The distribution is in classes which differ by one per cent, grouped on the nearest half per cent.

Per Cent Sucrose in Juice of Seedlings of Different Parentage Groups.

	9.5	10.5	11.5	12.5	13.5	14.5	15.5	16.5	17.5	18.5	19.5	20.5	Totals	Mean	Standard Deviation	Coefficient of variability
1912																
Otaheite parentage ..	..	..	..	..	2	2	6	3	1	1	6	2	23	17.72 ± .292	2.06 ± .206	11.6 ± 1.168
D—117 " ..	..	..	1	2	6	9	19	21	21	5	3	..	87	16.23 ± .117	1.62 ± .083	9.9 ± .510
1916																
D—418 parentage ..	1	1	0	0	3	4	6	11	8	4	..	..	38	15.97 ± .215	1.97 ± .152	12.4 ± .959
D—117 " ..	..	..	1	1	5	5	8	2	2	..	..	..	24	14.81 ± .196	1.43 ± .139	9.6 ± .934
Crys.×D—109 parentage ..	1	2	0	2	6	9	8	13	7	..	..	..	48	15.16 ± .181	1.89 ± .130	12.5 ± .873

There are some differences shown in the frequency distributions of these populations; however, seedling canes are very much affected by environmental influences, and no data are at hand to show that frequency distributions of subsequent generations of such groups would show the same relation as that shown here. Moreover, it is the individual seedlings that are of interest, as new varieties are formed by asexual multiplication of these; and even though we assume the above to be the case, we still cannot say that the chances of selecting superior seedlings are greater in a percentage group showing a relatively high frequency distribution. than a low one, until it is shown that the individual ranges of variation of subsequent generations of the separate seedlings of these groups bear a relation corresponding to that of these first generation seedlings. The coefficients of variability of these groups of seedlings range from 9.6 $\pm$ .934 of the D–117 parentage group of 1916, to 12.5 $\pm$ .873 of the Crystallina $\times$ D–109 parentage group, the latter being a little greater than that of the D–448 parentage group, which was 12.4 $\pm$ .959. Statistics of different years are probably not comparable, though it is notable that the coefficient of variability of the D–117 canes was the same for both years. In both years the coefficient of variability of the D–117 canes was the smallest, and in the 1916 seedlings those of D–448 and Crystallina $\times$ D–109 were about the same. The number of individuals in any group is not sufficiently large, and the data at hand are not sufficient to allow conclusions to be drawn. The table is included with the other data at hand at this time, mainly for the purpose of pointing out a line of work which may give results when it has been completed.

CONCLUSIONS.

1. Seedling sugar canes in their first generation show a degree of resemblance to the varieties from which they were produced.

2. The results of the work at this Station indicate that resemblance of color is more marked than that of any other characteristic.

3. There is wider variation in seedlings than in canes produced from cuttings of the same variety.

4. The greatest variation in seedlings produced from tassels of a single variety is in the size and form of the plants, and of their component parts.

5. Certain varieties produce better seedlings than others.

6. Abnormalities are common in seedling canes, whereas in canes

produced from cuttings they are rare. Certain varieties produce many more abnormal seedlings than others.

7. New types of cane are produced by crossing varieties.

8. Variation is apparently increased by a single combination of two varieties.

9. Crossing seems to produce a recombination of characters of the parents in some of the resulting seedlings, this probably being due in a measure, to dominance of certain characters derived from each parent.

10. Only slight differences in sugar content of the juice have been observed between groups of seedlings produced from different varieties.